The effect of different light quality treatments on plants pigment-protein complexes dynamics

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The composition and organization of pigment-protein complexes depend on environmental conditions, especially on the quality and quantity of the light. Different light quality changes the plant's sense and response to its environment. In order to understand the dynamics in the composition of the thylakoid membrane protein complexes under different light treatment, it is important to develop techniques allowing reliable separation of the protein complexes. BN-PAGE is an excellent tool for analyses of proteins and protein complexes in their native form, thus enabling the analysis of their subunit composition. Here, to reveal the different light quality-dependent protein interactions in the thylakoid network, we analyze the pigment-protein complexes using a BN-PAGE technique. Data presented here show that besides of the changes amount of different pigment-protein complexes, appear one new band in the blue-light treated thylakoid sample.

Keywords: *crop plants, thylakoid membrane, pigment-protein complexes, blue native gel electrophoresis, light-emitting diode, blue light, white light*

INTRODUCTION

In order to avoid photodamage under excess light intensities and to maximize light absorption during light deficiency plants have developed a series of strategies for light-harvesting regulation. Plants harvest light mainly via the light-harvesting complex (LHC) II and two large pigment-protein assemblies called photosystems (PSI)I and PSII, which are working in sequence to transform light energy into chemical energy and linked through the plastoquinone pool and the cytochrome b_6f complex (Wientjes et al., 2013). Both PSII and PSI are connected with peripheral LHC that accumulate photon energy to convert that energy into chemical energy in reaction centers of photosystems. While LHCI entirely transfers light energy to PSI, with which it is strongly associated (Croce and van Amerongen, 2014), the LHCII can transfer energy to PSII and/or PSI (Grieco et al., 2015; Longoni et al., 2015). PSII and its reaction center, where water oxidation in photosynthesis takes place, have been indicated as the primary target of photoinhibition (Vass et al., 1992). The interaction of pigment-protein complexes in PSII depends on environmental conditions, mostly on the quality and quantity of the light (van Amerongen and Croce, 2013). Changes in light intensity and wavelength can manipulate the plant metabolism and respond to their environment. It was shown that recent development of light-emitting diode (LED) technologies presents an enormous potential for improving plant growth and making systems more sustainable (Darko et al, 2014). LED light has the capacity to mimic the effect of natural light thus ensuring the growth and development of photosynthetic organisms. The impact of supplemental red and/or blue LED light is not limited to plant growth and developmental properties but even increases the antioxidant content of vegetables. For instance, investigation directed on the effect of red (658–660 nm) LED light to have shown the increase of the concentration of phenolic compounds in lettuce leaves (Li and Kubota, 2009) and the anthocyanin content of red cabbage leaves (Wua et al., 2007). The 470 nm of additional red or blue LED light caused stress whereby the xanthophyll cycle was activated whereas the effect of blue light has been shown less stressful in compare

with red light (Fu et al., 2013; Darko et al, 2014).

The investigation of the pigment-protein interaction under different light quality and quantity requires the detailed characterization of multiprotein complexes (MPCs) to obtain reliable data for the understanding protein function and regulation. The investigation of the dynamics of the thylakoid membrane protein complexes requires the development of the techniques with enhanced resolution capacity for separation of the protein complexes. BN (blue native)-PAGE, where anionic Coomassie Brilliant Blue dye is used to introduce a negative charge and visual stain for the proteins and protein complexes (Schägger and von Jagow, 1991; Heinemeyer et al., 2007), has been the widespread method for native-PAGE separation (Järvi et al., 2011). This technique that letting the separation of different MPCs according to their size and shape in a polyacrylamide gel is useful to determine the size, composition, and relative abundance of MPC (Schägger and von Jagow 1991; Schägger et al., 1994; Fiala et al., 2011).

In the present study, the effect of white LED light is compared to those of blue light (BLUE) treatment by a BN-PAGE technique to investigate the pigment-protein interactions in the thylakoid membrane of the rice plants.

MATERIALS AND METHODS

Plants and Growth Condition

One-month-old seedlings of WT rice (*Oryza sativa* L.) were grown on soil in a greenhouse (100 - 1700 μmol photons m^{-2} s^{-1} fluctuating light; 16-h photoperiod) at a temperature of 30/26 °C (day/night). The middle parts of the leaves were used for experiments.

Light treatment

For the light treatment, one-month-old seedlings of WT rice were kept in the dark for 12 h and then treated with white and blue light-emitting diodes (LEDs) of ~700 μmol photons m^{-2} $s^{-1}$for 10min. Treated samples were frozen for further use.

Sample preparation for BN-PAGE

Thylakoid isolation was performed under dim light at 4°C. Thylakoid membranes were isolated according to (Järvi et al., 2011) with some modifications from frozen leaves of WT dark and high light (~700 μmol photons m^{-2} s^{-1}) treated samples. Leaves were homogenized by mortar and pestle with liquid nitrogen and then grinded in ice-cold grinding buffer [50 mM Hepes/KOH (pH 7.5), 330 mM sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 5 mM ascorbate, 0.05% BSA and 10 mM sodium fluoride]. The homogenate was filtered through four layers of Miracloth followed by centrifugation at 5 500 g at 4°C for 6 min. The resulting pellet was washed and resuspended in a shock buffer [50 mM Hepes/KOH (pH 7.5), 5 mM sorbitol, 5 mM $MgCl_2$ and 10 mM sodium fluoride] and then centrifuged at 5 500 g at 4°C for 6 min. After, the pellet was resuspended in a storage buffer [50 mM Hepes/KOH (pH 7.5), 100 mM sorbitol, 10 mM $MgCl_2$,10 mM sodium fluoride] followed by centrifugation again at 5 500 g at 4°C for 6min. Finally, the thylakoid pellet was suspended into a small aliquot of storage buffer. The chlorophyll concentration was determined from the samples as described in (Porra et al., 1989).

The thylakoid membrane (8 μg of chlorophyll) was pelleted at 5 500 g at 4°C for 6 min, and the supernatant was discarded. The resulting pellet was resuspended into ice-cold 25BTH20G [25 mM BisTris/HCl (pH 7.0), 20% (w/v) glycerol, 10mM sodium fluoride]. An equal volume of detergent solution (diluted in 25BTH20G) was added to a final concentration of 1.0% (w/v) β-DM (Sigma-Aldrich). The thylakoid membrane was solubilized in darkness on ice for 5 min; insolubilized material was removed by centrifugation at 13 000 rpm at 4°C for 10 min. Prior to loading, the samples were supplemented a one-tenth volume of Serva Blue G buffer [100 mM BisTris/HCl (pH 7.0), 0.5 M ACA, 30%(w/v) sucrose and 50 mg · ml^{-1} Serva Blue G] to introduce a negative charge and to increase the solubility of the sample.

Preparation of BN-PAGE gel

Optimal separation of the thylakoid membrane protein complexes by BN-PAGE was obtained by using an acrylamide linear gradient of 4.5%–13.5% separation gel and 4% (w/v) stacking gel using gradient gel assembly (Amersham Biosciences; Little Chalfont Bucks, UK). The anode buffer [50 mM BisTris/HCl (pH 7.0)] and the cathode buffer contained [50 mM

Tricine, 15 mM BisTris] were used for BN-PAGE. Electrophoresis was performed by 10 mA at 4°C for 35-40 min. As the blue running front has moved about a half (at ~100-125 V) of the desired running distance, the cathode buffer which becomes blue-colored was removed from the upper chamber and replaced with a clear cathode buffer. The BN-PAGE was stopped when the Coomassie blue ran out of the bottom of the gel.

RESULTS AND DISCUSSION

To investigate the dynamics of the thylakoid membrane protein complexes under different light quality treatment the blue native polyacrylamide (BN-PAGE) technique allowing separation of the protein complexes under native conditions was used.

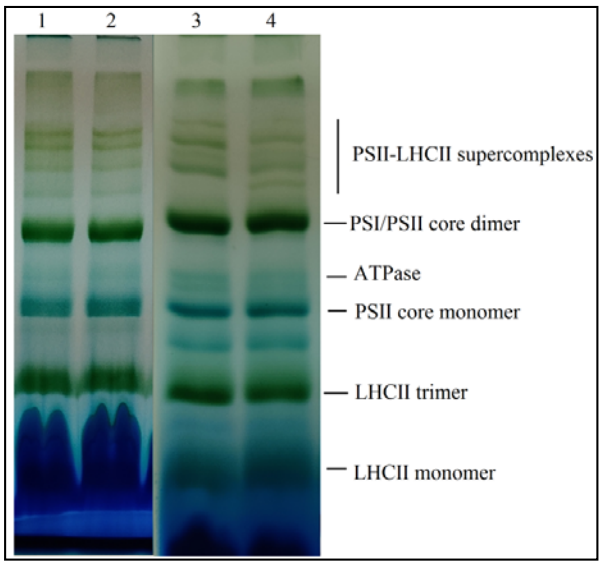

Fig. BN-PAGE analyses of thylakoid membrane protein complexes from WT rice (*Oryza sativa* L.) genotypes. Thylakoid membranes (8 µg of Chl) were solubilized with 1% DM and separated by BN-PAGE. 1–dark, 2–HL white LED light,3–dark, 4 –HL blue LED light

By using BN-PAGE we were able to separate 12 distinct bands in dark-adapted and white light treated rice leaves, and 13 bands in blue light treated leaves (Figure). After both white and blue LED light treatments there was a slight decrease in band intensities belonging to supercomplexes. Some differences between pigment-protein complexes distribution between white and blue light might be due to different sampling and different experiment time. While there were some differences between dark and white light treated samples, turning to the blue light treated samples, there was one more band belonging to supercomplexes. According to the published results, this band is the PSI-LHCII complex at state 2 (Crepin and Caffarri, 2015). The appearance of the additional band after blue light treatment might be due to the activation of cryptochrome (CRY), a blue-light photoreceptor (Ahmad et al., 2002; Darko et al., 2014). Also, there was a slight decrease in the band intensity belonging to ATPase in blue light treated samples. It has been shown by (Kohzuma et al., 2017) ATP synthase redox regulation may be impacting a number of cellular processes such as the accumulation of chloroplast proteins and/or ions or the reaction of photosynthesis to rapid changes in light intensity.

CONCLUSION

The BN-PAGE was shown to provide an invaluable tool to analyze the dynamics of the thylakoid membrane complexes upon changing light conditions. It was shown that so as the white LED light is close to natural light there were some negligible differences after treatment with ~700 µE light intensity for 10 min, while treatment with blue light has shown significant differences in comparison with dark-adapted samples.

Thus, research directed on the effects of LEDs on plant responses, coupled with advances in the dynamic modification of light quantity and quality in different phases of growth may contribute to the efficient utilization of LED lighting technologies in crop plants cultivation in closed environments.

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Müxtəlif işıq keyfiyyətinin bitkilərdə piqment-zülal komplekslərinin dinamikasına təsiri

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Bitki piqment-zülal komplekslərinin tərkibi və təşkili ətraf mühit şəraitindən, xüsusən işığ enerjisinin keyfiyyətindən və miqdarından asılıdır. Fərqli işıq keyfiyyəti bitkilərin həssaslığını və ətraf mühitə reaksiyasını dəyişir. Müxtəlif işıq keyfiyyəti və intensivliyi təsiri altında olan tilakoid membrane zülal komplekslərinin dinamikasında baş verən dəyişikliklər ianlamaq üçün zülal komplekslərinin etibarlı şəkildə ayrılmasına imkan verən texnikaların inkişaf etdirilməsi zəruridir. BN-PAGE zülal və zülal komplekslərini native formada analiz etməyə və bununla da subunit tərkibini təhlil etməyə imkan yaradan üsuldur. Tilakoid membranlarda fərqli işıq keyfiyyətinə bağlı zülalların qarşılıqlı təsirlərini öyrənmək üçün, piqment-zülal kompleksləri BN-PAGE üsulundan istifadə etməklə analiz olunmuşdur. Təqdim olunan məlumatlar göstərmişdir ki, fərqli piqment-zülal komplekslərinin miqdarında izlənən dəyişikliklərlə yanaşı, mavi işıq təsirindən tilakoid nümunələrində yeni bir zolaq əmələ gəlir.

Açar sözlər: *Dənli bitkilər, tilakoid membranı, piqment-zülal kompleksləri, mavi nativ gel elektroforez, işıq emissiya edən diod, mavi işıq, ag işıq*